Claims 1 and 2 also stand rejected as allegedly lacking novelty in view of the disclosure of WO 88/01058, published February 11, 1988.

Claim 3 has also been rejected under 35 U.S.C. §103 as allegedly obvious based on the disclosure of WO 88/01058, considered in view of the commercial availability of the Bio-Rad Lasersharp MRC 500.

Claims 1 and 2 have been further rejected under 35 U.S.C. §103 as allegedly obvious based on the combined disclosures of WO 84/01031 and the above-noted Chen et al. patent. According to the Examiner, it would have been obvious to a person of ordinary skill in the art at the time this invention was made to modify the methods of WO 84/01031 by the inclusion of immobilized labelled binding members for the purported advantages disclosed in the Chen et al. patent.

Claims 1-3 have also been rejected under 35 U.S.C. §103 as allegedly unpatentable over the combined disclosures of WO 84/01031 and the Chen et al. patent, as applied to claims 1 and 2 above and further in view of the commercial availability of the Bio-Rad Lasersharp MRC 500. The Examiner asserts in this regard that in view of the advantages of confocal miscroscopy it would have been obvious to one of ordinary skill in the art at the time the present invention was made to have performed the claimed method employing a laser scanning confocal microscope, such as the Bio-Rad Lasersharp MRC 500.

The Examiner has also made an additional reference of record, which is deemed by the Examiner to be exemplary of the level of ordinary skill in the art prior to the time the present invention was made with respect to confocal microscopy. Specifically, White et al., J. Cell. Biol., 105: 41-48 (1987) has been made of record, but no rejection has been based on this particular reference.

With regard to the two separate grounds of rejection based on alleged obviousness-type double patenting, applicant is submitting herewith a Terminal Disclaimer in proper form in

accordance with the provisions of 35 U.S.C. §253 and 37 C.F.R. §1.321(b) and (c). Applicant's Terminal Disclaimer is made with reference to the '099 patent, is signed by the attorney of record, as permitted under Rule 321, and is accompanied by the appropriate fee.

It is settled law that a timely filed Terminal Disclaimer under 35 U.S.C. §253, which satisfies the requirements of 35 C.F.R. §1.321(b) and (c), will overcome an "obviousness-type" double patenting rejection.

The present application is designated as a continuation-in-part of applicant's U.S. Application No. 07/984,264, filed December 1, 1992 ("the parent application"), which, in turn, is a continuation of applicant's U.S. Application No. 07/460,878, filed February 2, 1990 ("the grandparent application"). The grandparent application claims the benefit of the priority date of applicant's British Application No. 8803000, filed February 10, 1988. This claim of convention priority is appropriate in the present application and is hereby asserted herein. A certified copy of the priority document is in the official file of the grandparent application. See §201.14(b) of the Manual of Patent Examining Procedure.

Inasmuch as applicant is entitled to the benefit of his priority filing date of February 10, 1988 under the International Convention, as provided under 35 U.S.C. §119, the prior art rejections based variously on the Ekins article and WO 88/01058, either individually or in combination with other references, cannot be maintained, given that the publication dates of both the Ekins article and WO 88/01058 are after February 10, 1988.

The only grounds of rejection remaining to be addressed are the §§103 rejections of claims 1 and 2 based on WO 84/01031 and the Chen et al. patent, and of claims 1-3 based on the same two references, as well as the commercial availability of the Bio-Rad Lasersharp MRC 500. These last-mentioned grounds of rejection are respectfully traversed.

The rejections under §103 based on the combined disclosures of WO 84/01031 and the Chen et al. patent (as to claims 1 and 2), with additional reference on the commercial availability of the Bio-Rad Lasersharp MRC 500 (as to claim 3) are plainly improper for the reasons that:

(1) Ekins does not teach the use 0.1 V/K moles of binding agent as called for in applicant's claims; and

(2) The Chen et al. patent cannot properly be combined with WO 84/01031 in the manner proposed by the Examiner. WO 84/01031 and Chen et al. are concerned with different types of assay, and there would have been no motivation or incentive for the person of ordinary skill in the art to combine them, nor any way in which they can be easily combined.

1. The Assay of WO 84/01031 Does Not Use Less Than 0.1 V/K Moles of Binding Agent

WO 84/01031 concerns an assay wherein results which are sample-volume independent are obtained using a small amount of binding agent that does not significantly alter the concentration of the analyte in the sample.

However, WO 84/01031 defines the amount of binding agent used operationally and does not define it in terms of physicochemical parameters (e.g., V,K) as in the present case. As can be seen in the examples set forth in WO 84/01031, the amount of binding agent used in the samples having volumes 0.2, 0.4 and 0.8 ml can be calculated to give the amounts of binding agent equal to V/K, 0.5 V/K and 0.25 V/K, respectively. These amounts are all in considerable excess of the more stringent requirements of this application.

It would not have been obvious that any advantage can be obtained by reducing still further the amount of binding agent to provide response for claim 1; after all, it would at first sight seem that this would result in a reduction in the signal obtained and make the assay less sensitive. However, applicant's assay can in fact be just as

sensitive at the levels of binding agent now claimed (0.1 V/K or even a great deal smaller).

One distinct advantage of the assay of the invention which is not disclosed or suggested in WO 84/01031 and which clearly is not obvious from WO 84/01031 is that the use of an amount of binding agent less than 0.1 V/K moles results in a small amount of analyte being removed from the total, irrespective of analyte concentration, (i.e., on the flat portions of the curves in the figure accompanying the present application). This is certainly not the case in WO 84/01031, which only requires the amount of binding agent not to significantly deplete the ambient concentration of analyte, i.e., the small amount used in WO 84/01031 has necessarily to be related to the expected analyte concentration. WO 84/01031, therefore, has the disadvantage that the person carrying out an assay described therein has to know at least approximately the expected concentration of the analyte in the sample. This is a problem where the concentration of an analyte varies over a considerable range, e.g., the *in vitro* concentration of the hormone HCG varies from around 0.1 to 100 or more IU/ml, see page 10, lines 30 to 32 of the present application.

In the present invention, only the volume of the sample and the affinity constant of the binding agent need to be known to calculate a suitable amount of binding agent. As the Examiner will appreciate, this is an apparent practical advantage over WO 84/01031.

Therefore, WO 84/01031 does not lead the person of ordinary skill towards the present invention and does not disclose the advantages of the claimed invention.

It has long been recognized that silence in a reference is not a proper substitute for adequate disclosure of facts from which a conclusion of obviousness may justifiably follow. *In re Burt*, 148 U.S.P.Q. 548 (CCPA 1966).

2. The Chen et al. Patent Discloses an Assay

Fundamentally Different From That of WO 84/01031 and Thus Cannot Properly be Combined with WO 84/01031 so as to Render the Present Invention Obvious

As noted by the PTO Board of Appeals in Ex parte Wolters, 214 U.S.P.Q. 735 (Bd. Apps. 1979), the burden of establishing a *prima facie* case of obviousness falls upon the Examiner. In determining whether a case of *prima facie* obviousness exists, it is necessary to ascertain whether or not the disclosures of the cited prior art would appear to be sufficient to one of ordinary skill in the art to make the claimed substitution, combination or other modification. *In re Lalu*, 223 U.S.P.Q. 1257 (Fed. Cir. 1984). Merely because it is possible to find two prior art disclosures which might be combined in such a way as to arrive at the claimed subject matter does not make the combination of disclosures obvious unless the art also contains something to suggest the desirability of the proposed combination. *In re Imperato*, 179 U.S.P.Q. 730 (CCPA 1973).

In the present case, there is nothing to suggest the desirability of combining the disclosures of WO 84/01031 and the Chen et al. patent in the manner proposed by the Examiner.

The assay of the Chen et al. patent is conventional in the sense that the amount of receptor must be kept constant because, for any given analyte concentration, the fraction of the binding sites occupied by analyte varies with the amount of receptor present. Therefore, if the analyte is labelled as envisaged by Chen et al. and the receptor were also labelled, **the ratio of the signals emitted by the bound analyte and by the receptor would be dependent on the amount of receptor present**.

In the conventional assays of the prior art (which does not include WO 84/01031) it is usual to try to bind virtually all the analyte present in a sample. This means that doubling the amount of receptor present will double the signal emitted by the receptor. However, as no more analyte

will be bound, the analyte signal would remain constant. **Consequently, the ratio of the two signals would not be related to the concentration of the analyte in the sample**.

The Examiner relies on the Chen et al. patent for its purported suggestion of dual labelling in a solid phase assay. There is no dispute that dual labelling is reasonably well known in the art, but for quite different reasons than its use in the present invention. This observation applies to the assay of the Chen et al. patent as well as any other conventional, prior art assay employing dual labels.

Examples of the prior art uses of dual labelling are the quality control and instrument calibration procedures disclosed by Chen et al., both of which are not relevant to the problem the present application successfully addresses of designing an assay which is independent of the amount of receptor used.

It is respectfully submitted that the Examiner has failed to identify any reason why the person of ordinary skill in the art would wish to use dual labelling, for the purposes disclosed by Chen et al., in combination with the method of WO 84/01031. Although there are a wide variety of possible uses of dual labelling, there is no disclosure or other motivation for the skilled person to use dual labelling to produce an assay which is independent of the amount of receptor, i.e., one in which it does not matter if the amount of receptor varies.

With regard to quality control, Chen et al. discloses the labelling of the antibody to ensure that the correct amount of antibody has been attached to the substrate and that the resulting product has not been damaged in transit (page 4, line 30 through page 5, line 5). This procedure is exemplified in Example 1 of Chen et al. and simply involves measuring the strength of the signal emitted by the labelled antibody. Those samples where the signal is abnormally high or abnormally low are discarded (compare page 11, lines 17 and 18).

The only other purpose for dual labelling of the antibody taught by Chen et al. is so that the signal emitted by the labelled antibody can be quantitatively detected independently of the detection of the labelled ligand bound to it. In this way, according to Chen et al., the disclosed immunoassay procedures may be made "self-calibrating" (see page 5, lines 6-10). By "self-calibrating", Chen et al. are, however, referring to the calibration of the fluorometer and not the calibration of the immunoassay by comparison with standard samples. In this embodiment, according to its preferred form, a fluorescent tag on the antibody and a fluorescent tag on the back-titration reagent are detected quantitatively while they are bound to each other, using the same fluorometer, and the quantity of ligand present in an unknown sample is determined as a function of the ratio of the quantitative measurements of the two tags. Any instrumental error or defect affecting the measurement of the strength of the fluorescent signal, particularly effecting the gain of the surface to fluorometer interface will affect both signals equally, according to Chen et al. and will leave the ratio unaffected (see page 9, lines 12-24). This aspect of the procedure is exemplified by Chen et al. in Example 2 and the tables on pages 14 and 15, where two different types of background surface are compared, namely, clear glue and black glue, and measurements are given to show that the determination of the ratio of tag measurements can compensate for variable gain in the interface between the sampler and the fluorometer (page 12, lines 31-34). However, because Chen et al. are operating a conventional immunoassay procedure and not one in which only a trace amount of antibody is used, it remains vital to Chen et al.'s procedure to assume that the amount of labelled antibody exposed to each test sample is absolutely constant when ratios are being measured, otherwise a variation in the antibody signal from sample to sample will be misinterpreted as a variation in the light-measuring efficiency of the fluorometer which Chen et al. are attempting

to eliminate. There is simply no motivation provided to those skilled in the art by the teaching of Chen et al. to employ dual labelling under the specific conditions that characterize the assay of the present invention.

From an objective reading of WO 84/01031 and Chen et al., it is clear that if one attempts to combine either of the foregoing teachings of Chen et al. with the assay of WO 84/01031 one would be led to label the antibody in order to serve either as quality control of the levels of antibody on the substrates to be exposed to the samples, or as a means of avoiding or compensating for instrument error. One would not, however, be led to realize the fundamental inventive step of the present invention, namely, the fact that under the special conditions of WO 84/01031, it is not necessary that the amount of antibody exposed to each sample need be the same. This is a fundamental difference and one which forms the basis for a new "ratiometric" assay procedure which was not known or evident to those skilled in the art before the present invention. It was certainly not known or evident from Chen et al., which has no relevance to the calculation of the binding cites.

Viewed from another perspective, it cannot reasonably be considered obvious to combine the disclosure of Chen et al. and WO 84/01031 to solve the problem addressed by the present invention, because both prior art assays have that problem inherent within them. This is explained further as follows.

The Examiner should not underestimate the importance of the problem of immobilizing receptor in a known amount, or an amount known to be constant, on a support. Before the present invention, it was considered imperative to do this. This can be seen as WO 84/01031 uses an amount of receptor known, or known to be constant, while Chen et al. actually measure the receptor present by labelling and discards those that do not have the specified amount.

In reality, commercial manufacturers have spent and

continue to spend, large amounts of time, effort and money developing materials and methods suitable for accurately depositing receptor on supports. In the past, problems existed regarding the topology of the surface of the support, the materials from which the support is made and the method of fixing the receptor to the support. The applicant's achievement bypasses these problems with the present invention, since it is not necessary to know the amount of the receptor, or even for it to be constant.

Thus, it should be abundantly clear that there can have been no incentive for the skilled person to combine WO 84/01031 and Chen et al. to solve this problem, since they both retain this requirement as regards the receptor, and therefore their combination can hardly have suggested its elimination.

A clear advantage that the present invention provides is that in the conditions described in the applicant's earlier application (WO 84/01031), the ratio of the signals from markers on the receptor and the back-titration reagent is functionally linked to the fractional occupancy of the receptor. This relationship was not recognized in the prior art documents cited by the Examiner, as WO 84/01031 does not use two markers and the assay of Chen et al. does not use a small amount of receptor (i.e. Chen et al. do not use, or remotely contemplate using, an amount of receptor that only has an insignificant effect on the concentration of analyte being measured).

As noted above, Chen et al. use a double-label procedure whose purposes are to reassure the user of an immunoassay kit that the same standard amount of antibody is present on all the test surfaces and/or to obviate errors arising from a change in the efficiency of the signal measuring equipment (e.g. fluorometer). In other words, the double label according to Chen et al. is used specifically to ensure that the amount of immobilized receptor is constant, and to compensate for possible variations in the measurement

efficiency. The present invention is not concerned with these factors.

Variations in measurement efficiency are not a serious problem in practice, while in the present invention the need to know that the amount of receptor is constant is completely obviated.

With reference to the above comments on WO 84/01031, it is apparent that the proposed combination of WO 84/01031 in view of Chen et al. does not teach or suggest an assay which is independent of the amount of receptor, as neither of these references individually discloses this clearly desirable goal. Chen et al. does not make up for the deficiency of WO 84/01031 noted above. The Examiner's arguments at page 5 of the Official Action, concerning the combination of WO 84/01031 and Chen et al., are therefore, fatally flawed. Given that WO 84/01031 does not teach or suggest a way of solving the problem of making an assay independent of the amount of receptor, this feature alone distinguishes the present invention from the prior art in a way which is both novel and non-obvious.

It is the invention as a whole that must be considered in determining obviousness under 35 U.S.C. §103. The focus of inquiry under §103 is clearly not limited solely to the actual structure or operative steps embodying the claimed invention. Rather, relevant properties or advantages of the invention must also be taken into account in an appropriate case. As aptly noted in *In re Antonie*, 195 U.S.P.Q. 6, 8 (CCPA 1977):

> In delineating the invention as a whole, we look not only to the subject matter which is literally recited in the claim in question . . . but also to those properties of the subject matter which are inherent in the subject matter and are disclosed in the specification. [Emphasis in original].

To the same effect is *In re Estes*, 164 U.S.P.Q. 519 (CCPA 1976) (advantages accruing from claimed process need not be recited; such advantages should be considered in determining patentability).

Moreover, the particular problem facing the inventor must also be taken into account in properly assessing obviousness under §103. *In re Rinehart*, 189 U.S.P.Q. 143, 149 (CCPA 1976).

None of the art of record is concerned with the problem which applicant has successfully solved in accordance with the present invention, namely, providing an assay that is independent of the amount of receptor used. Such an assay affords a distinct practical advantage over assays available heretofore, which is neither achievable by the assays of the cited prior art references, nor suggested by the disclosures thereof. References which do not recognize applicant's problem cannot have suggested its solution. *In re Schaffer*, 108 U.S.P.Q. 326 (CCPA 1956).

The present invention uses dual labelling in a new way in combination with the sample-volume independent assay of WO 84/01031 to produce an assay which is independent of both the sample volume and the amount of receptor uses. The use of dual labelling in this way has not been proposed before and furthermore is not disclosed or suggested by Chen et al.

Chen et al. merely disclose the labelling of receptor to facilitate quality control, or to use the ratio of the signals between receptor and analyte to account for factors affecting the optical gain of surfaces having different characteristics.

Furthermore, there is no actual suggestion in WO 84/01031 or Chen et al. directing the person of ordinary skill to make the combination of these references at all. The mere simultaneous existence of features disclosed by two prior art references does not suggest modifying one reference to produce the claimed invention. *Ex parte Hoyt*, (BBPA 1992).

As the foregoing discussion makes clear, there is a

fundamental difference in the way the assays of Chen et al. and WO 84/01031 address the problem of obtaining a sensitive assay, the assay of Chen et al. using a large amount of binding agent to try to bind all the analyte in a sample, whereas the assay of WO 84/01031 represents a sampling technique. There is no motivation for the person of ordinary skill in the art to make the proposed combination.

Therefore, the Examiner's argument here appears to rely on hindsight in that it ignores the state of the art at the priority date by suggesting a combination of references that would not have been perceived by the person of ordinary skill in the art in 1988 as leading to any useful result.

Turning to the rejection of claims 1-3 based on WO 84/01031, the Chen et al. patent and the commercial availability of the Bio-Rad Lasersharp MRC 500, obviousness cannot be established on such a combination in the absence of some indication or teaching or incentive to the person of ordinary skill to combine the elements from the cited prior art. *In re Geiger*, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987). As mentioned above in relation to the combination of WO 84/01031 and Chen et al., applicant was the first person to realize that an assay which is independent of the amount of receptor could be carried out using a small amount of receptor, in combination with dual labelling.

Moreover, none of the references disclose an assay which is independent of the amount of receptor. Thus, even if the combination of the disparate elements from the cited prior art were made, recognition of this unique and patentable feature would still be lacking. Consequently, claims 1-3 cannot reasonably be held obvious in light of the cited prior art.

As note previously, the White et al. article was cited in the October 16, 1996 Official Action, but was not applied against any of the claims. That being the case, no detailed discussion of this reference would appear to be in order. Suffice it to say that the White et al. article fails

to provide evidence of lack of novelty or obviousness with respect to the subject matter of claims 1-3.

It is respectfully requested that the requirement for corrected drawings, which accompanied the October 16, 1996 Official Action, be held in abeyance, pending the indication of allowable subject matter.

In view of the Terminal Disclaimer submitted herewith and the foregoing remarks, it is respectfully urged that all of the rejections of record be withdrawn and that this application be passed to issue, and such action is earnestly solicited.

Respectfully submitted,

DANN, DORFMAN, HERRELL AND SKILLMAN
A Professional Corporation

By Patrick J. Hagan
PATRICK J. HAGAN
PTO Registration No. 27,643

Telephone: (215) 563-4100
Facsimile: (215) 563-4044

Enclosure: Terminal Disclaimer